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Flowering, capsule and seed characteristics in *Cuphea*

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Abstract We modeled the flowering and capsule set dynamics, quantified the level of variation in seed characteristics, elucidated the inter-relationships between seed and capsule physical dimensions, and quantified their impact on single seed weight in the cuphea germplasm line PSR23. Temporal patterns of flowering and capsule set were largely time-dependent and highly auto-correlated, with significant auto-regressive parameters of number of flowers (0.76) and number of capsules (0.74). Large numbers of seeds per capsule were associated with large coefficient of variation in single seed weight. Seed number and seed weight per capsule, when log-transformed, exhibited a slope of −0.65 indicating that cuphea plants have only a limited capacity to maintain seed weight by adjusting seed number if resources vary. Capsule tissue/seed weight ratio and number of seeds per capsule have the largest potential impact on single seed weight. Sensitivity analyses indicated that capsule perimeter (>76 mm), capsule major (>23 mm) and minor axes (>7.0 mm), capsule circularity (between 0.350 and 0.375), capsule area (<85 mm^2), capsule tissue/seed weight ratio (<0.40), and number of seeds per capsule (∼9.0) would optimize single seed weight. This information would help plant breeders exploit genotypic variability in seed and capsule characteristics and agronomists identify optimum trait combinations to produce high yields of this potential oilseed crop.

Keywords Phenolgy · Flower dynamics · Capsule dynamics · Seed characteristics · Sensitivity analysis

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Introduction

Flowering, temporal distribution of flowers, pollination, seed set, and seed and capsule characteristics are among the most important factors determining seed yield in the cuphea germplasm line PSR23, a potential oilseed crop selected from an inter-specific cross between two species of the Lythraceae: *Cuphea lanceolata* and *C. viscosissima* (Knapp and Crane 2000). Flower production and seed set are key, but not well understood, phases in the yield production process of crop plants (Egli 2005), especially in indeterminate plants such as cuphea. The continued production of nodes during flowering and capsule set plays a role in determining the total duration of flowering of cuphea plants; in addition, flower production profiles are highly influenced by environmental factors (Vega et al. 2001; Egli 2005); therefore, they may be amenable to experimental manipulation.

In indeterminate crop plants, the critical period for seed set extends from anthesis to beginning or middle seed filling (Vega et al. 2001). Empirical

evidence (Turnbull et al. 1999) supports the association between seed number and plant growth; however, the putative relationship between seed number and plant growth rate during the critical period for seed number determination is not unique but strongly depends on the reproductive strategy of the crop. One of the problems facing breeders and agronomists of cuphea PSR23 is that it flowers over a very long period; therefore, capsule number may exhibit a non-linear saturation response as documented in indeterminate wild plants (Lutmann 2002) and crops (Egli 2005). In addition, large levels of reproductive failure can occur at several stages of reproductive development due to abortion and abscission (Hiei and Ohara 2002; Egli 2005) of both flowers and immature ovules.

Cuphea seed size and weight are inherently non-uniform because of the presence of multi-ovules within a non-uniform capsule (Graham 1989). Seed size is an important component of life history in plants (Koelewijn and van Damme 2005), as even a small variation may influence seedling emergence, seedling growth and survival, seedling competition and yield. However, due to the strong stabilizing selection on seed size (Turnbull et al. 1999), individual plants that produce seeds either smaller or larger than the optimum may suffer reduced fitness and consequently any observed variability in seed size may be maladaptive.

The number of harvestable seed per unit area, and seed oil content are the dominant yield components in many oilseed crops (Gambín and Borrás 2005); therefore, variation in seed size contributes greatly to final seed and oil yield determination. A keener insight into the regulation of seed size, seed number, and determination of seed yield is possible if flowering and seed set dynamics in cuphea are well understood. However, gaining this understanding proved to be difficult in indeterminate crops (Egli 2005) because the biological system in these crops is complex and it is not readily amenable to experimental manipulation.

The objectives of this study were: to (1) model the flowering and capsule set dynamics, (2) quantify the level of variation in seed characteristics, (3) elucidate the inter-relationships between seed and capsule physical dimensions and (4) quantify their impact on single seed weight as a determinant of seed yield in cuphea PSR23.

Materials and methods

Field experiments

Experimental plots, arranged in a randomized complete block design with four replicates, were established in 2004 and 2005 on a Barnes-Buse loam (Barnes fine-loamy, mixed Udic Haploboroll, Buse fine-loamy, mixed, Udorthentic Haploboroll) at the Swan Lake Research Farm located near Morris, MN (45°41′ N, 95°48′ W, elevation 370 m). The field site was previously in corn and soybean for the 2004 and 2005 experiments, respectively. Planting (14 kg seed ha^{-1}; May 19, 2004 and May 17, 2005) and fertilizer application (110, 12 and 30 kg ha^{-1} of N, P and K, respectively) were done mechanically at a depth of about 15 mm. Seed produced in 2003 and 2004 were used for the 2004 and 2005 field experiments, respectively. Each plot consisted of six rows (6 m long and 60 cm row spacing); the middle two rows were mechanically harvested (September 28, 2004 and September 16, 2005) for seed yield determination. Weed control was carried out to ensure a weed-free stand. At physiological maturity, six single plants were randomly sampled from each replicate for detailed morphological (plant height, number of main branches, plant dry weight, LAI, and fractal dimension) and agronomic (capsule and seed characteristics, and seed yield per plant) evaluation (Table 1). Number of flowers and capsules were monitored during 2004 and 2005 on $plant^{-1}$ and m^{-2} basis, respectively, and number of seeds per plant was estimated from seed dry mass data at harvest.

The flowering and fruiting (i.e., capsule formation) phenology of PSR23 was monitored during 2004 and 2005 growing seasons. Number of flowers and capsules on 10 plants (in 2004) and in 1 m^2 (in 2005) were censured in four replicates throughout the flowering and capsule formation period. Six single plants were randomly sampled from each replicate at physiological maturity for detailed morphological and agronomic evaluation and number of seeds per plant was counted at harvest. During the 2004 and 2005 cropping seasons, approximately 200 mature random capsules were weighed individually, then seed weight and seed number per capsule were obtained. The ratio between seed weight and the capsule tissue weight (i.e., packaging cost) was calculated for each capsule. A scale (in mm) was attached to each object (capsule

Table 1 Descriptive statistics of eight traits measured or estimated on plants of the cuphea germplasm line PSR23 and averaged over two (2004–2005) growing seasons

Trait	Mean	Standard deviation	Coefficient of variation	Percent variance due to years ($P < 0.05$)
Plant height (cm)	76	14.7	19.3	24.9
Above ground biomass (gm^{-2})	970	270	27.9	27.0
Flowers (m^{-2})	1060	593	56.0	77.5
Scaled flowers g^{-1} biomass	1.142	0.429	37.9	
Capsules (m^{-2})	1245	991	79.6	83.6
Scaled capsules g^{-1} biomass	1.174	0.739	62.9	
Reproductive failure	42.8%	12.6	29.4	
Potential seed yield ($kgha^{-1}$)	1,240	860.3	69.4	

or seed lot), and the generated image was saved as a digitized 8-bit gray image with a resolution of 300 pixels. For each scanned image, all measurements were adjusted based on the pixel-to-mm conversion scale. Potential seed number per plant (PSNP) was estimated as the product of total number of flowers and average number of seeds per capsule and reproductive failure was calculated as the ratio of filled capsules to total number of flowers (Vega et al. 2001).

Statistical analyses

All statistical analyses procedures were implemented using modules in the STATISTICA software package (StatSoft Inc. 2005a) unless otherwise specified. Descriptive statistics were developed for eight traits measured or estimated on plants, capsules and seeds, and averaged over two cropping seasons (Table 1). Autocorrelation functions in the time series module were calculated for flowers m^{-2} and capsules m^{-2} and the Box-Ljung's Q-test of autocorrelation was used to verify the significance of the time-series analyses of number of flowers and number of capsules. The Q-test of autocorrelations determines whether a given count (i.e., number) of open flowers or mature capsules is dependent on its previous count or whether it depends on time only. Non-linear regression models using the Non-linear module were developed to quantify the dynamics of flowering and capsule formation in response to growing degree days or as functions of plant biomass.

Digital imagery (Adamsen et al. 2000; Rasband 2004) and analysis procedures (Fourotan-pour et al. 2000) were used to capture, and measure morphological traits of individual capsules and seeds (i.e., area, perimeter, major axis as seed length, and minor axis as seed width; Table 2). Individual capsules and their seed content were manually positioned on a platform ensuring seeds were totally separated from each other, then digitally photographed using a Nikon D70 digital camera with a 1,504 by 1,000 pixels resolution. The number of seed in each digital photo was verified by the number of objects generated by ImagJ software program (Rasband 2004). A step-wise clustering procedure in the Cluster module (StatSoft Inc. 2005a) separated the seed produced in 2004 and 2005 into three categories (i.e., small, medium and large, with average seed weight of 2.4, 3.1 and 3.5 mg, respectively) with significant differences among these categories for four seed characteristics (Table 2).

The impact of capsule and seed variables, including nutrient density (i.e., concentration) in $\mu g\ g^{-1}$ of seed dry weight, on single seed weight was studied using feed-forward, back-propagation artificial neural networks (ANNs) module (StatSoft Inc. 2005a). ANNs models were subjected to sensitivity analysis to evaluate the relative importance of each variable in explaining variation in single seed weight. In this analysis, each predictor was treated in turn as if it were not available in the ANN model and the average value of that predictor was used.

A sensitivity ratio was calculated by dividing the total ANN error when the predictor was treated as "not available" by the total ANN error when the actual value of the predictor was used. If the ratio is >1.0, then the predictor made an important contribution to 1000-seed weight or to seed yield m^{-2}; the higher the ratio, the more important is the predictor (StatSoft Inc. 2005b). Additionally, we calculated the correlation coefficient (r) and a ratio between the standard deviation (SD-ratio) of original and model data; higher r values and lower SD-ratio values are indicators of better model performance (StatSoft Inc. 2005b). Sensitivity analysis was performed by

Table 2 Mean separation for seed characteristics and proportion of variance accounted for by differences between small, medium, and large seed categories of the cuphea germplasm line PSR23 and averaged over two (2004–2005) growing seasons

Seed variable	Seed weight category			Percent variance
	Small	Medium	Large	
Average seed weight (mg)	2.4c*	3.1b	3.5a	
Area (mm^2)	6.432c	6.767b	6.958a	20
Perimeter (mm)	9.511c	9.723b	9.989a	25
Major axis (length) (mm)	3.22b	3.23b	3.34a	10
Minor axis (width), mm	2.61c	2.66b	2.69a	26
Circularity, % (ratio of minor axis/major axis)	0.882a	0.891a	0.884a	5

* Means of a seed variable followed by the same letter do not differ significantly (Tukey HSD, $P = 0.05$)

generating response curves for each predictor to study its relationship with single seed weight, while all other predictors were set at their mean value.

Results

Descriptive statistics

Descriptive statistics for four measured (plant height, biomass m^{-2}, flowers and capsules m^{-2}), three derived (scaled number of flowers and number of capsules, and reproductive failure) and one estimated variables (potential seed yield, gm^{-2}) indicate that PSR23 is a heterogeneous population (Table 1). On average, plants were 76 cm tall, and produced 970 g of biomass, 1,060 flowers and 1245 capsules m^{-2}. When number of flowers and number of capsules were scaled (per unit of biomass), a plant is expected to produce 1.142 and 1.174 flower and capsule per g of biomass, respectively. Nevertheless, the reproductive failure (42.8%) was large and may have contributed to the small average potential seed yield of 1,240 kg ha^{-1}.

Coefficients of variation (C.V.) were large for most variables (ranged from 19.3 for plant height to 79.6 for capsules m^{-2}); however, the C.V. estimates were extremely large for capsules m^{-2} (79.6%) as compared to flowers m^{-2} (56.0%), and for potential seed yield g m^{-2} (69.7%) as compared to biomass g m^{-2} (27.9%). A sizable portion of total variation in each of four measured variables was accounted for by differences between the two growing seasons; it was large for flowers and capsules m^{-2} (77.5 and 83.6%, respectively), and relatively small for plant height and biomass m^{-2} (24.9 and 27.0%, respectively).

Dynamics of flowering and capsule set

Autocorrelation functions for log(flowers m^{-2}) and log(capsules m^{-2}) and test statistics of the correlation coefficients between time lags in PSR23 are presented in Fig. 1. The Box-Ljung's Q-test of autocorrelation and Barlett's test verified the significance of the time-series analyses of log(flowers m^{-2}) and log(capsules m^{-2}). The Q-tests of autocorrelation indicate that a given count (i.e., number) of open flowers or formed capsules at time t + 1 were dependent on their previous counts at time t, and not on time only. Correlation coefficients were lag-dependent and decreased in magnitude (and in sign, positive or negative) as the lag between successive counts increased.

Flower initiation and capsule formation on the indeterminate PSR23 plants progressed linearly up the main stem and on secondary branches as a function of thermal time (Fig. 2a). Dynamics of flowers and capsules m^{-2} in response to growing degree days (Fig. 2a), and scaled number of flowers and number of capsules as functions of plant biomass (Fig. 2b) indicate that capsule number, in both cases, exhibited a non-linear saturation response; the quadratic portions of all four regression equations were highly significant ($P < 0.01$). Intercepts and slopes of the linear and quadratic components for the prediction of flowers and capsules m^{-2} as a function of GDD (Fig. 2a) and as a function of biomass g m^{-2} (Fig. 2b) indicated that time after flower initiation and the plant capacity to accumulate biomass have antagonistic effects on these two variables. Two inflection points

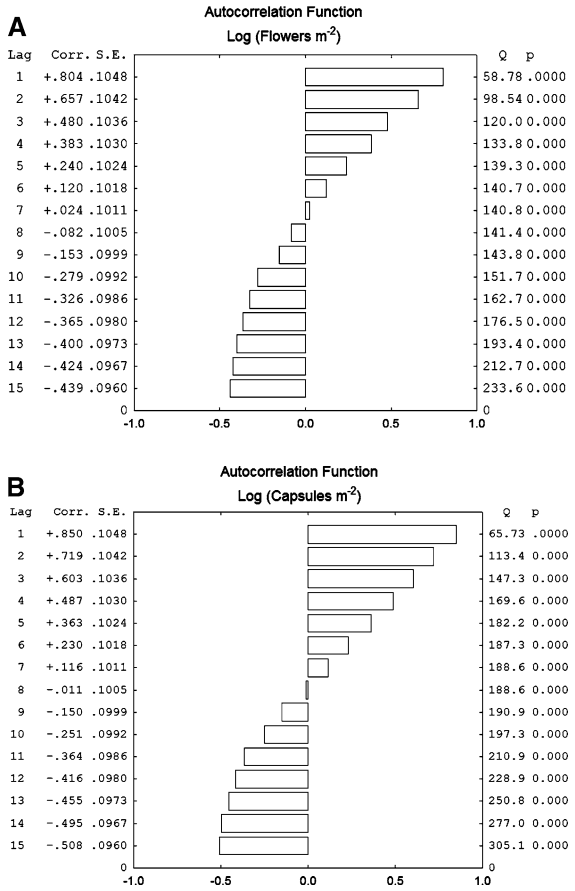

Fig. 1 Autocorrelation functions for log flowers m^{-2} (**a**) and log capsules m^{-2} (**b**) and test statistics of the correlation coefficients among the first 15 lags in the cuphea germplasm line PSR23 monitored over two growing seasons (2004–2005)

were observed at about 800 GDD (and ~400 g biomass m^{-2}), and at ~ 1,000 GDD (and ~900 g biomass m^{-2}), and a highly significant correlation was found (Fig. 2a) between these two variables ($r = 0.82$, $P < 0.001$).

The autoregressive parameter estimates of number of flowers (0.76) and capsules (0.74) per plant were highly significant ($P < 0.001$); however, these estimates were associated with large 95% confidence intervals (0.55–0.97 and 0.52–0.94, respectively), and a large portion of variance in number of flowers (77.5%, $P < 0.01$) and capsules (83.6%, $P < 0.01$) was attributed to differences between years. Nevertheless, number of flowers and number of capsules per plant can be estimated with reasonable accuracy as a function of thermal time ($R^2 = 0.79$, and 0.86, respectively).

Seed characteristics

Seed weight categories (Table 2) displayed normal distributions and their skewness and kurtosis statistics were not significant, except for circularity, which is the ratio between minor (i.e., seed width) and major (i.e., seed length) axes. Seed dimensions and the variance explained by differences between small, medium and large seed weight categories indicate that the differences were highly significant between all three categories for seed area, perimeter and minor axis with significant R^2 values >20.0; however, large seed differed from small and medium seed in major axis, and there were no significant differences between all three categories in seed circularity; a small and not significant portion of variance in these two seed characteristics (10.0 and 5.0%, respectively) was attributed to differences between seed weight categories. Seed circularity was almost constant although there were significant differences between seed weight categories for seed major and minor axis.

Joint variation of capsule and seed characteristics

Correlation coefficients between seed and capsule dimensions (Table 3) ranged from positive and significant between area, perimeter and major axes of capsules and seeds, to negative and non-significant between those involving minor axes and circularity. However, all positive correlation coefficients were <40.0, and the negative ones ranged from −0.06 to −0.21. The canonical R between seed and capsule dimensions was 0.76 [χ^2 (64 d.f.) = 62.5, $P = 0.53$], and the amount of variation explained in seed dimensions given capsule dimensions (i.e., total redundancy) was 22.5% (Fig. 3). However, when seed weight and seeds per capsule were included in the canonical regression analyses, canonical r increased to 0.85 [χ^2 (80 d.f.) = 87.9, $P = 0.25$], and the amount of variation explained in seed dimensions given capsule dimensions increased to 51.9%.

Determinants of seed number and seed weight

The regression model expressing the relationship between number of seed per plant and plant dry weight was not significant ($R^2 = 0.17$); however, the quality of the fit of the data to the regression models was improved ($R^2 = 0.38$) by analyzing the loga-

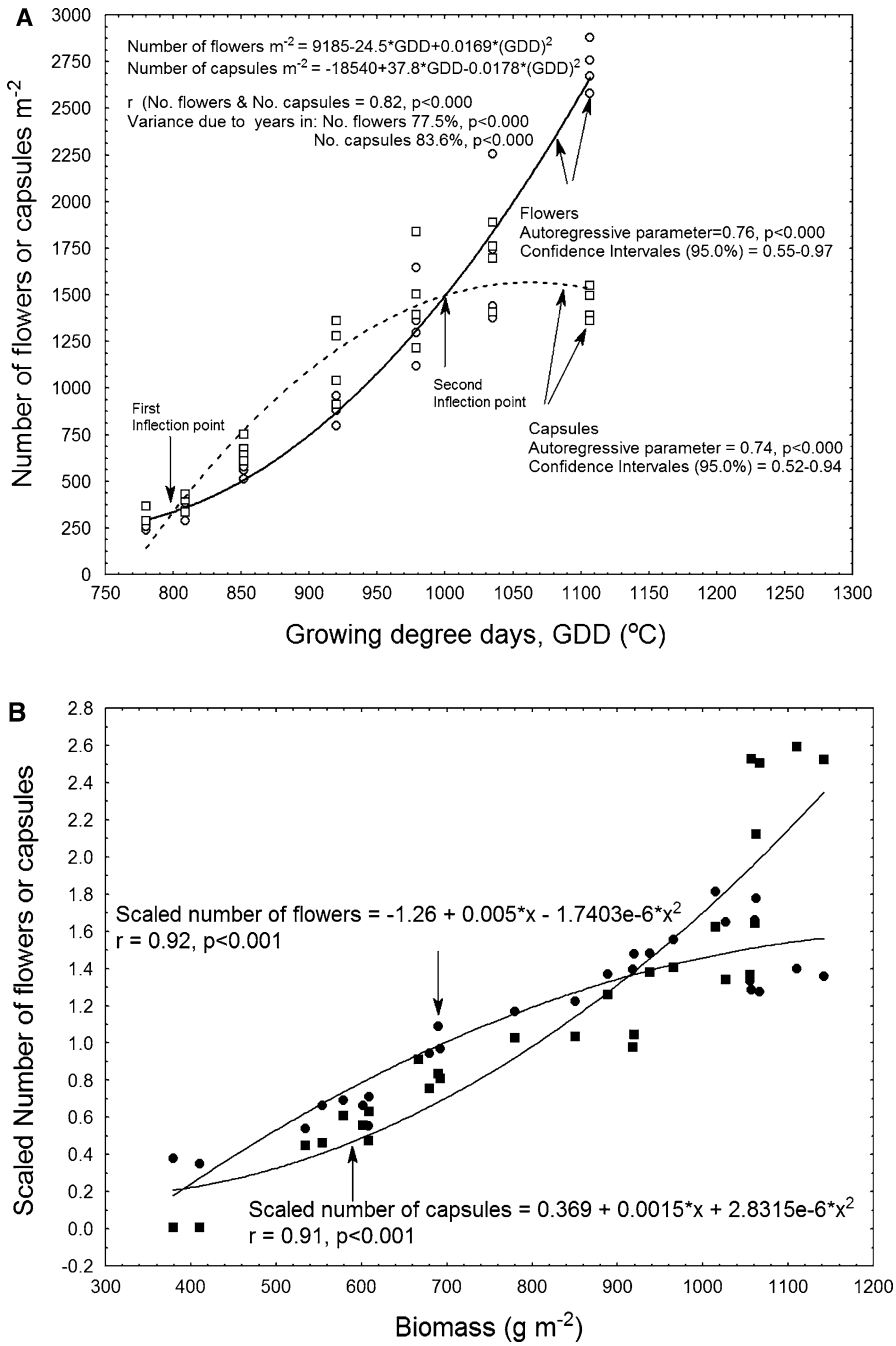

Fig. 2 Dynamics of number of flowers and number of capsules of the cuphea germplasm line PSR23 in response to growing degree days (**a**), and scaled number of flowers and number of capsules as functions of plant biomass (**b**) monitored over two growing seasons (2004–2005)

rithms of both seed numbers and plant dry weights data not presented). On the other hand, a negative relationship ($r = -0.79$, $P < 0.001$) was found between log(seed number) and log(seed weight) as expressed by the regression model: log(seed weight) = -1.078–$0.65 \times$ log(seed number); however, when adjusted for plant dry weight, a slightly larger correlation coefficient ($r = -0.87$, $P < 0.001$) was obtained. The negative slope (-0.65) was significantly different from an inverse relationship of -1.0 ($t = 9.7$, $P < 0.05$).

A negative correlation ($r = -0.78$, $P < 0.001$) was found between mean seed weight and the coefficient of variation (C.V.) of single seed weight based on the

Table 3 Correlation coefficients between seed and capsule dimensions of the cuphea germplasm line PSR23 averaged over two (2004–2005) growing seasons

Seed/capsule	Area	Perimeter	Major axis	Minor axis	Circularity
Area	0.35	0.37	0.33	0.18 ns*	−0.18 ns
Perimeter	0.38	0.40	0.35	0.18 ns	−0.20 ns
Major axis	0.37	0.39	0.32 ns	0.20 ns	−0.19 ns
Minor axis	0.32 ns	0.32 ns	0.29 ns	0.17 ns	−0.13 ns
Circularity	−0.21 ns	0.30 ns	−0.28 ns	−0.06 ns	0.25 ns

* ns = not significant ($P > 0.05$), otherwise $P < 0.05$

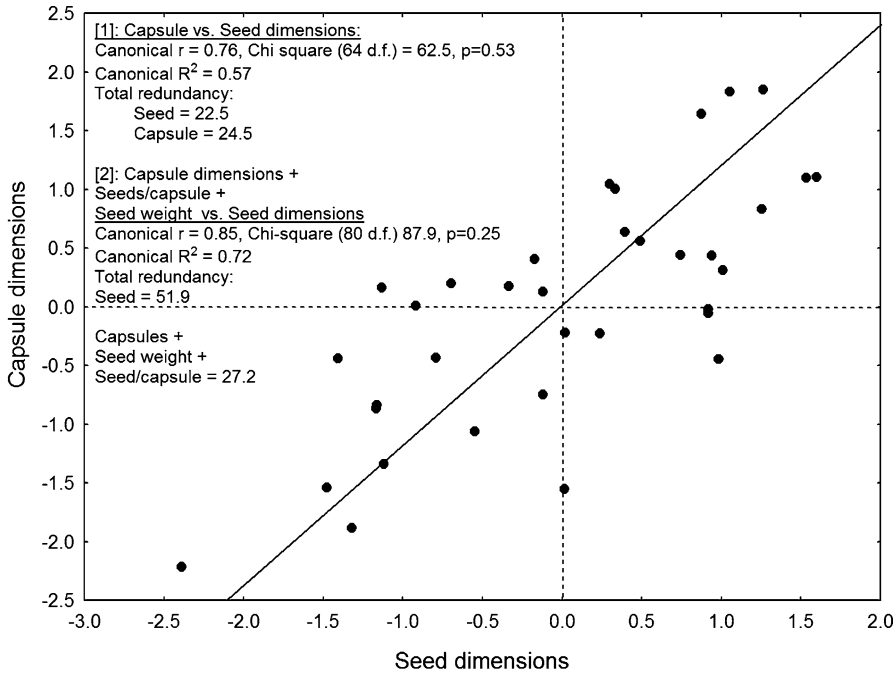

Fig. 3 Canonical correlation between seed and capsule dimensions of the cuphea germplasm line PSR23 measured on seeds extracted from ~200 mature capsules during 2004 and 2005 growing seasons

regression model: C.V. of single seed weight = 35.6–4.9*mean seed weight, and only 60.0% of the variation in the former was explained; however, when the number of seeds per capsule was included in the regression model, a much larger portion of variation in C.V. of seed weight (91.0%) was explained. Mean number of seeds per capsule ranged from 5 to 17, the C.V. of single seed weight ranged from ~10.0 to 26.0%, and the larger C.V. values were generally associated with larger numbers of seeds per capsule.

Differences in nutrient densities (μg g^{-1} seed weight) when normalized, explained a small portion ($R^2 = 0.27$) of the variation in log(single seed weight) (Fig. 4); however, the relationship was negative and significant ($r = -0.52$, $P < 0.01$), and when combined with log(plant dry weight) (Fig. 5), explained 47.0% of variation in log(number of seed per plant). The multiple regression model indicates that log(dry plant weight) had a positive impact; whereas the normalized nutrient density had a negative, albeit smaller and decreasing impact on log(number of seed per plant) as log(plant dry weight) increased.

Determinants of single seed weight

A three-dimensional plot based on the correlation matrix between 14 seed and capsule characteristics (Fig. 6) separated these characteristics along the three axes and formed four relatively independent groups in relation to their impact on single seed weight. Seed area, perimeter, major axis and minor axis, along with seed weight per capsule and log(nutrient density)

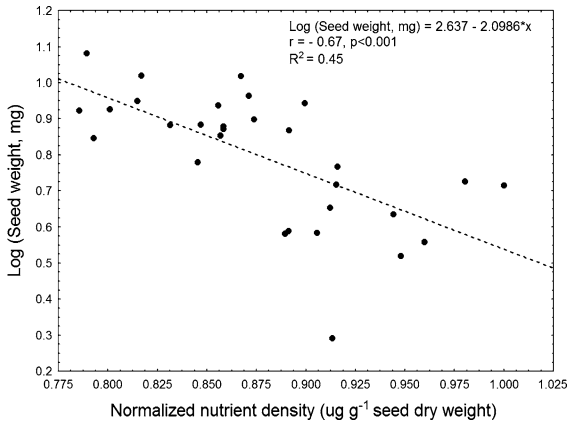

Fig. 4 Relationship between normalized nutrient density (μg g^{-1} of seed dry weight) and log (seed weight, mg) measured on seeds of the cuphea germplasm line PSR23 sampled during two (2004–2005) growing seasons

formed the first group and were positively associated (except for the last two traits) with single seed weight on the second and third dimensions. Seed circularity was the only seed characteristic not to be associated with single seed weight. The second group was composed of capsule weight, area, perimeter, and major axis, in addition to number of seed per capsule, all of which, except the last characteristic were positively associated with single seed weight on the third dimension. The third group, composed of seed circularity, capsule circularity and capsule minor axis, was separated from single seed weight on the first two dimensions, and finally the fourth group which was composed of capsule minor axis and capsule circularity, was positively associated with single seed weight on the third dimension.

Number of seeds per capsule followed in decreasing order by capsule area, capsule minor axis, capsule tissue/seed weight ratio (i.e., packaging cost), and capsule major axis were the most important characteristics influencing single seed weight as indicated by their ratio and rank in a neural network analysis (Table 4). The remaining characteristics, especially capsule perimeter with a ratio <1.0, did not exert the same impact on single seed weight. Reliability of the neural network analysis was tested by comparing several test statistics of the training and test samples (Table 4). The mean and the S.D. of single seed weight were larger for the test samples (3.378 and 1.03, respectively) as compared to the training samples (3.315 and 0.65, respectively). However, the S.D. ratio (0.23 and 0.18, respectively) and coefficient of determination (0.89 and 0.94, respectively), as the

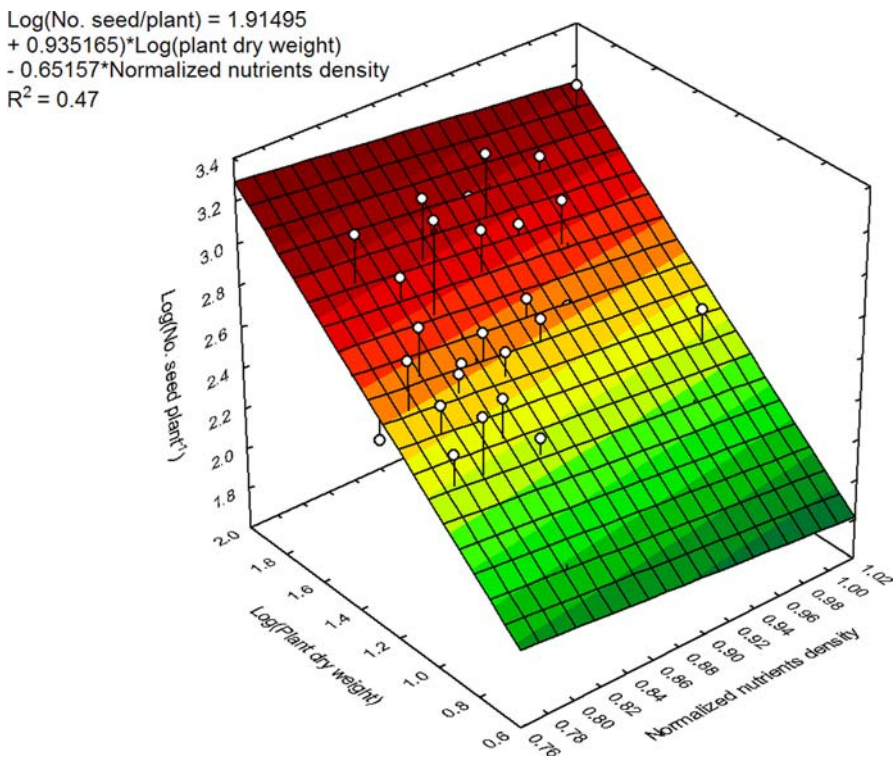

Fig. 5 Log (number of seed per plant) as a function of log(plant dry weight) and normalized nutrient density (μg g^{-1} of seed dry weight) measured on seeds of the cuphea germplasm line PSR23 sampled during two (2004–2005) growing seasons

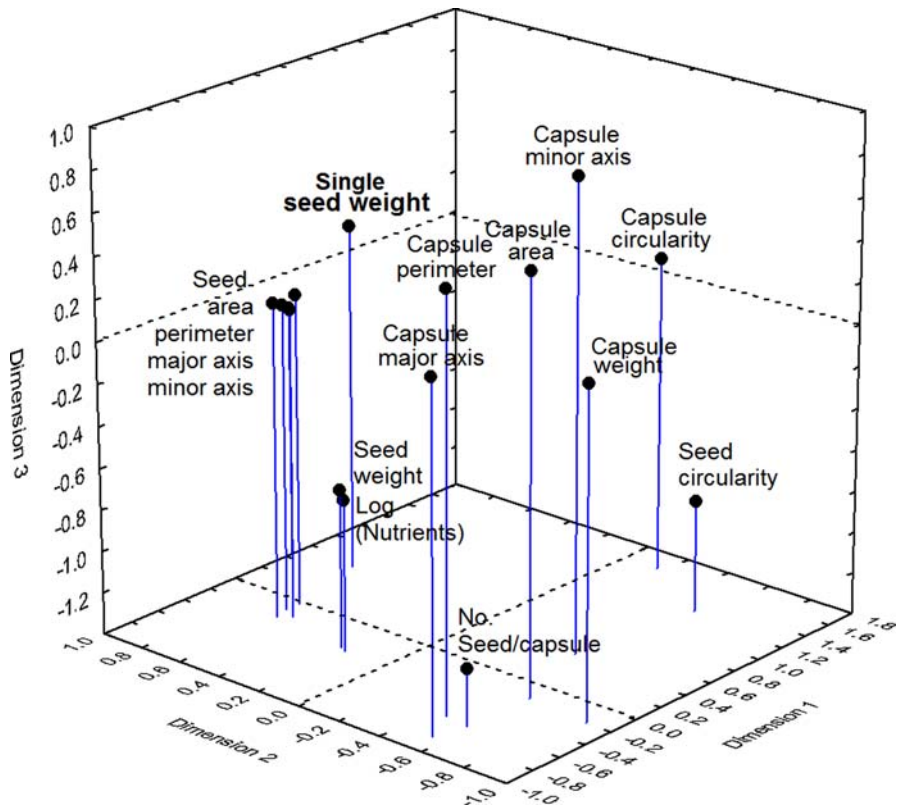

Fig. 6 Three-dimensional plot based on the correlation matrix between seed and capsule characteristics measured on single seeds extracted from ~200 capsule of the cuphea germplasm line PSR23 during two (2004–2005) growing seasons

Table 4 Neural Network statistics (ratio, rank and test statistics) for important variables influencing single seed weight of the cuphea germplasm line PSR23 during two (2004–2005) growing seasons

Independent variable	Dependent variable single seed weight, mg		Test statistics	
	Ratio	Rank		
Seeds capsule^{-1}	2.39	1		
Capsule/seed weight ratio	1.88	4		
Normalized nutrients density	1.07	7		
Capsule area	2.34	2		
Capsule perimeter	0.96	8		
Capsule major axis	1.5	5		
Capsule minor axis	2.11	3		
Capsule circularity	1.19	6		
			Training sample	Testing sample
Mean			3.315	3.378
Standard deviation (S.D.)			0.65	1.03
Coefficient of variation (C.V.)			19.6	30.5
S.D. Ratio			0.18	0.23
Coefficient of determination, R^2			0.94	0.89

most important test statistics, were comparable for both samples.

Sensitivity analyses of single seed weight (Fig. 7) identified eight independent variables between all 14 seed and capsule characteristics as statistically important in determining single seed weight. Positive linear and significant ($P < 0.01$) effects were displayed by capsule perimeter ($r = 0.85$), capsule circularity

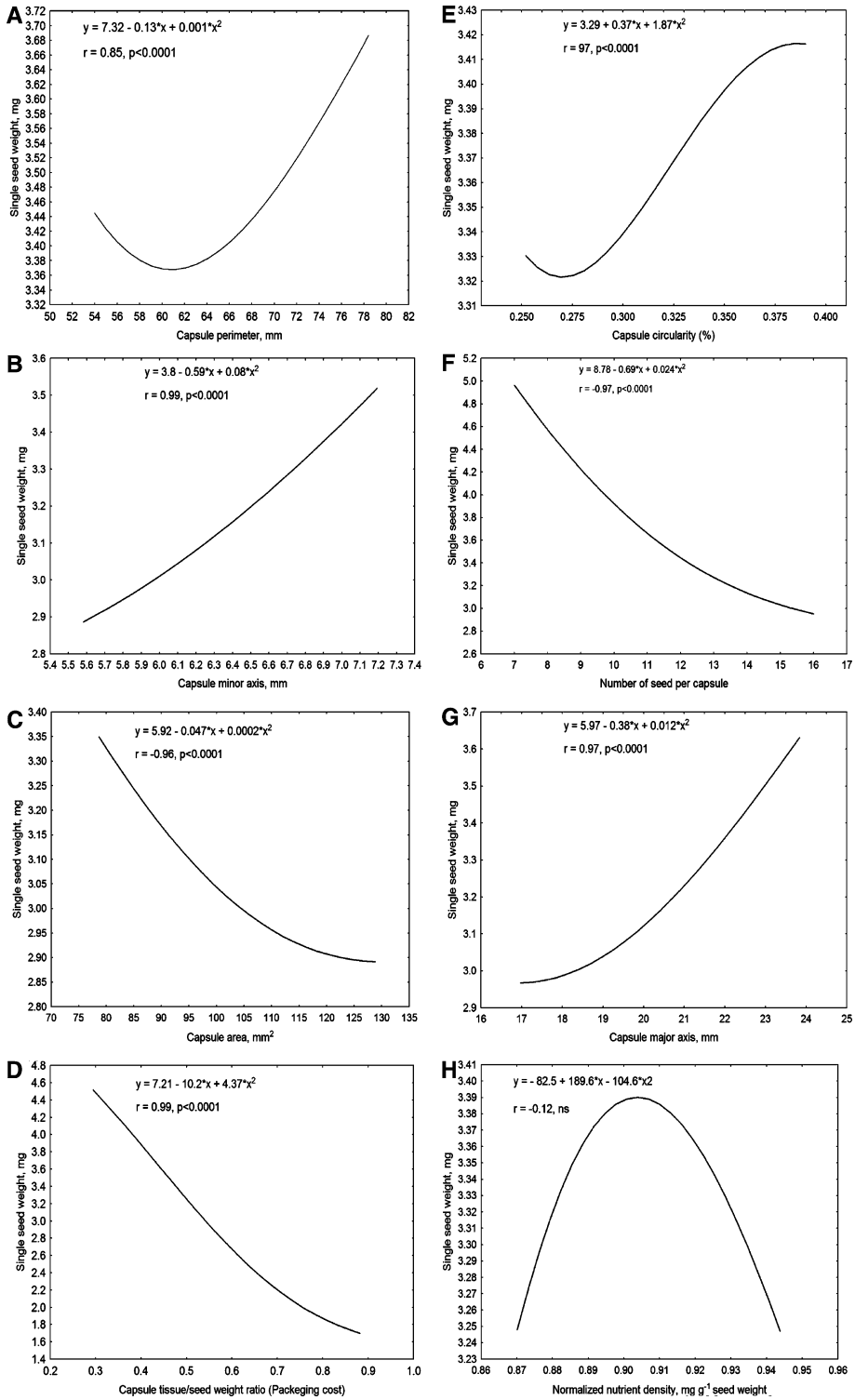

Fig. 7 Sensitivity analysis of single seed weight as a function of eight variables (see Table 4) averaged over two (2004–2005) growing seasons and based on a Neural Network model developed for the cuphea germplasm line PSR23

($r = 0.97$), capsule major axis ($r = 0.97$), and capsule minor axis ($r = 0.99$); however, the non-linear component in the regression equations was significant only for capsule perimeter and capsule circularity.

Negative and significant ($P < 0.01$) linear correlation between single seed weight were displayed by capsule area ($r = -0.96$), capsule tissue/seed weight ratio ($r = -0.99$), and number of seeds per capsule ($r = -0.97$). The normalized nutrient density displayed a highly non-linear (bell-shaped) and non-significant ($r = -0.12$) correlation with single seed weight. Two segments can be identified in the regression curve; the first with a positive effect on single seed weight as normalized nutrient density increased to a maximum of 0.90; the second with a negative effect when the normalized nutrient density exceeded 0.91.

Minimum and maximum values on the y-axes (Fig. 7) indicate that capsule tissue/seed weight ratio (i.e., packaging cost) and number of seeds per capsule have the largest potential impact on single seed weight as compared to the relatively small potential impact of the remaining characteristics, especially capsule perimeter and normalized nutrient density. Maximum values of capsule perimeter (>76 mm), capsule major (>23 mm) and minor axes (>7.0 mm), and capsule circularity between 0.350 and 0.375 would optimize single seed weight; whereas, minimum values of capsule area (<85 mm), capsule tissue/seed weight ratio (<0.40), and number of seed per capsule (<9.0), in addition to a normalized nutrient density between 0.89 and 0.91 would optimize it.

Discussion

Descriptive statistics

A considerable phenotypic variation was found for all phenological traits, especially flowers and capsules (Table 1), whether expressed as C.V. or as percent variance due to annual variation. Therefore, understanding and modeling the dynamic nature of flowering and capsule set will require a more thorough understanding of the growth, biomass accumulation, development and abortion of individual capsules and the interaction between capsule and plant height and biomass (Egli 2005). Moreover, a thorough investigation of flowering dynamics is warranted as variation in the number of flowers may have a much greater effect than variation in capsule set on final seed production (Torres et al. 2002).

Dynamics of flowering and capsule set

Flowering and capsule set in PSR23 proved to be a dynamic process—spread out in time at individual nodes, mainly on the main stem, and on individual plants. We documented temporal patterns of flowering and capsule set as a function of thermal time (Egli 2005); however, much less is known about the regulation of these patterns and their involvement in determining number of filled capsules, number of seed per capsule, average seed weight, and yield.

The significance and potential value of the long flowering period of PSR23 is negated (Torres et al. 2002) by the fact that flowers produced late in the flowering period, due to the indeterminate growth habit of the plant, may abort before producing viable seed. Flower initiation progressed linearly up the branches as a function of thermal time; however, the number of flowers per plant may increase without a subsequent increase in the number of capsules (Hiei and Ohara 2002) when thermal time exceeded 1,000 GDD (Fig. 2a). Capsule abortion simply may increase if more flowers are produced without an increase in the potential productivity of the environment (Egli 2005). We observed that the fruit set within an inflorescence decreased in later flowers near the top; data presented by Hiei and Ohara (2002) on *M. roseum* supports our observation and was attributed to non-uniform pollination, resource competition, or excess flowers per plant and excess ovules per flower.

Seed characteristics

Cuphea seed is composed of the embryo, the cotyledons (absorbed by the embryo early during its development) and seed coat (Graham 1989). Each of these three structures is genetically distinct and may be affected by a number of interacting exogenous and indigenous factors. Cuphea seed weight is inherently non-uniform because of the presence of multi-ovules within a non-uniform capsule. A capsule of PSAR23 may contain 5–25 seeds and a seed (1.6–4.8 mg) may range in length (i.e., major axis) from 2.8 to 3.9 mm and in width (i.e., minor axis) from 2.4 to 2.9 mm, with the capsule tapering towards both ends, thus

affecting dimensions and weight of distal seeds. Similar non-uniformity in seed weight of *Lesquerella fendleri* (Brahim et al. 1998), oats (Doehlert et al. 2004), and sorghum (Gambín and Borrás 2005) were reflected on seed dimensions.

Joint variation of capsule and seed characteristics

Capsule characteristics, in addition to seed shattering, may contribute as secondary factors to the very low (4–14%) harvest index in PSR23 (Gesch et al. 2003; Sharratt and Gesch 2004). Capsule tissue/seed weight ratio (range from ~30 to ~85%) is among the largest as compared, for example, to wild and domesticated lupins (range from 24.6 to 77.7%) (Clements et al. 2005). Domestication tends to increase seed weight on the expense of the "packaging cost" and a concomitant increased indehiscence of pods, capsules, or siliques (Gepts and Papa 2002). Therefore, it could be feasible to genetically combine reduced-capsule shattering, larger seed weight and thinner capsules in cuphea as was demonstrated in lupins (Clements et al. 2005).

Seed area and seed perimeter are expected to increase as capsule area, perimeter and major axis increase; whereas, seed minor axis and circularity appear to be independent of changes in capsule dimensions. Capsule and seed dimensions may be independently manipulated to increase seed weight and seed yield as indicated by the non-significant to weak and significant ($r = 0.33$–0.40) correlation coefficients (Table 4). However, this may prove to be difficult considering the large canonical correlation coefficient ($r = 0.76$), especially when adjusted for seed weight and for seeds per capsule (canonical $r = 0.85$; Fig. 3).

Determinants of seed number and seed weight

Seed number per capsule and seed weight, both exhibiting considerable phenotypic variation, are expected to have an inverse relationship (Henry and Westoby 2001) because within a given reproductive production, increased provisioning for individual seeds implies a decrease in number of seed produced as demonstrated in this study. However, the evidence of a strong stabilizing selection on seed weight (Turnbull et al. 1999) is illustrated by the negative slope (-0.65, significantly different from -1.0) of log(seed number) on log(seed weight) per capsule, indicating that cuphea plants have only a limited capacity to maintain seed weight by adjusting seed number if resources vary (Henry and Westoby 2001). In addition, normalized nutrient density (Fig. 4) helped explain additional variation in number of seeds per plant, supporting the idea (Henry and Westoby 2001) that mineral nutrients constitute a fundamental cost for seed production over and above seed weight, and that some of the variation around the seed number-seed weight regression line could be explained by differences in seed nutrient density.

Apparently, seeds from capsules with small, medium, or large number of seeds in cuphea, unlike *Banksia marginata* seeds (Vaughton and Ramsey 1998) were not equivalent nutrient sinks. Additionally, C.V. of seed weight within a capsule, as a measure of resources distribution among seeds (Obeso 2004), indicates that the larger the number of seeds per capsule, the more heterogeneously resources are distributed among these seeds, and the larger the C.V.

Determinants of single seed weight

Seed weight is one of the least plastic of plant characteristics (Vaughton and Ramsey 1998). Nevertheless, we documented seed weight variation in PSR23, which is often pronounced within individual capsules and individual plants. This seed weight variation is inconsistent with empirical evidence (Gepts and Papa 2002) of strong directional selection favoring large seeds. Variation in seed position within the fruit (i.e., capsule) (Gambín and Borrás 2005) seed abortion (Hiei and Ohara 2002; Obeso 2004) and seed packaging costs (expressed as capsule tissue/seed weight ratio, Fig. 7), as investments in capsule tissue that provide protection and nutrition to the seeds (Obeso 2004), may contribute to seed weight variation.

We concur with Egli (2005) in that understanding and modeling the dynamic nature of flowering and capsule set in PSR23 will require a more thorough understanding of the growth, development and abortion of individual flowers, capsules and their interactions. However, this may prove to be difficult because cuphea, as a semi-domesticated, indeterminate crop, presents a complex system and may not be readily amenable to experimental manipulation unless components of its domestication syndrome (i.e., indeterminacy and seed shattering) are genetically

addressed. Nevertheless, the new knowledge we presented on its flowering and capsule set dynamics will provide a better insight into the regulation of seed number, the primary yield component, and therefore into the determination of its seed yield.

Conclusions

Number of open flowers and formed capsules in the cuphea germplasm line PSR23 were time-dependent, biomass-dependent, and highly auto-correlated. Large variations were observed in seed weight, and seed and capsule physical characteristics due mainly to the indeterminate growth habit of the plant. Seed size and weight are inherently non-uniform because of the presence of multi-ovules within a non-uniform capsule. Seed weight can be optimized by selecting plants with large capsule perimeter, capsule major and minor axes, capsule area, and small capsule tissue/seed weight ratio, and few (~9.0) seeds per capsule. These results suggest that flowering and capsule formation system in cuphea PSR23 germplasm line is complex and may not be readily amenable to experimental manipulation unless components of its domestication syndrome (i.e., indeterminacy and seed shattering) are genetically addressed. However, any knowledge of its dynamics will provide a keener insight into the regulation of seed number, the primary yield component, and therefore into the determination of seed yield. This information would help plant breeders exploit genotypic variability in seed and capsule characteristics and agronomists identify optimum trait combinations to produce high yields of this potential oilseed crop.

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References

Adamsen FJ, Coffelt TA, Nelson JM, Barnes EM, Rice RC (2000) Method for using images from color digital camera to estimate flower number. Crop Sci 40:704–709

Brahim K, Ray DT, Dierig DA (1998) Growth and yield characteristics of *Lesquerella fendleri* as a function of plant density. Ind Crops Prod 9:63–71

Clements JC, Dracup M, Buirchell BJ, Smith CG (2005) Variation for seed coat and pod wall percentage and other traits in a germplasm collection and historical cultivars of lupins. Aust J Agric Res 56:75–83

Doehlert DC, McMullen MS, Jannink J-L, Panigrahi S, Gu H, Riveland NR (2004) Evaluation of oat kernel size uniformity. Crop Sci 44:1178–1186

Egli DB (2005) Flowering, pod set and reproductive success in soya bean. J Agron Crop Sci 191:283–291

Foroutan-pour K, Dutilleuil P, Smith DL (2000) Effect of plant population density and intercropping with soybean on the fractal dimension of corn plant skeletal images. J Agron Crop Sci 184:89–100

Gambín BL, Borrás L (2005) Sorghum kernel weight: growth patterns from different positions within panicle. Crop Sci 45:553–561

Gepts P, Papa R (2002) Evolution during domestication. Encyclopedia of life sciences, Macmillan Publishers Ltd., Nature Publishing Group, pp 1–7/http://www.els.net

Gesch RW, Forcella F, Barbour NW, Voorhees WB, Phillips B (2003) Growth and yield response of cuphea to row spacing. Field Crops Res 81:193–199

Graham SA (1989) *Cuphea*: a new plant source of medium-chain fatty acids. Crit Rev Food Sci Nutr 28:139–173

Henry ML, Westoby M (2001) Seed mass and seed nutrient content as predictors of seed output variation between species. OIKOS 92:479–490

Hiei K, Ohara M (2002) Variation in fruit- and seed-set among and within inflorescences of *Melampyrum roseum* var. *japonicum* (Scrophulariaceae). Plant Species Biol 17:13–23

Knapp SJ, Crane JM (2000) Registration of reduced shattering cuphea germplasm PSR23. Crop Sci 41:299–300

Koelewijn HP, Van Damme JMM (2005) Effects of seed size, inbreeding and maternal sex on offspring fitness in gynodioecious *Plantago cornopus*. J Ecol 93:373–383

Lutman PJW (2002) Estimation of seed production by *Stellaria media, Sinapis arvensis* and *Tripleurospermum indodorum* in arable crops. Weed Res 42:359–369

Obeso JR (2004) A hierarchical perspective in allocation to reproduction from whole plant to fruit and seed level. Pers. Plant Ecol Syst 6:217–225

Rasband W (2004) ImageJ. 1.33a National Institute of Health, USA. http://rsb.info.nih.gov/ij/java1.5.0-beta

StatSoft Inc. (2005a) STATISTICA (data analysis software systems) Version 7.1 http://www.statsoft.com

StatSoft Inc. (2005b) Electronic statistics textbook, Tulsa. http://www.statsoft.com/textbook/stathome.html (last accessed 3 July, 2007)

Torres E, Iriondo JM, Pérez C (2002) Vulnerability and determinants of productivity success in the narrow endemic *Antirrhinum microphylum* (Scrophulaiaceae). Am J Bot 89:1171–1179

Turnbull LA, Rees M, Crawley MJ (1999) Seed mass and competition/colonization trade-off: a sowing experiment. J Ecol 87:899–912

Vaughton G, Ramsey M (1998) Sources and consequences of seed mass variation in *Banksia marginata* (Proteaceae). J Ecol 86:563–573

Vega CRC, Andrade FH, Sadras VO, Uhart SA, Valentinuz OR (2001) Seed number as a function of growth. A comparative study in soybean, sunflower and maize. Crop Sci 41:748–754